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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Markl et al.

Serial No.: 09/699,243

Filed: 27 October 2000

For: METHYLATION ALTERED DNA SEQUENCES AS MARKERS ASSOCIATED WITH HUMAN CANCER

Examiner: Jeanine A. Goldberg

Art Unit: 1634

Docket No.: 47675-14

Date: 24 January 2007

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF DR. KURT BERLIN UNDER 37 C.F.R. § 1.132
(IN SUPPORT OF RESPONSE UNDER 37 C.F.R. § 1.111)

Sir:

I, Dr. Kurt Berlin, being duly sworn, say:

1. I am familiar with the above-identified patent application, and am aware that it discloses and claims novel diagnostic methods comprising determination of the methylation state of CpG dinucleotide sequences that are differentially methylated between or among genomic DNA samples, including, for example between or among genomic DNA samples corresponding to normal and abnormal (*e.g.*, diseased, cancerous, hyperplastic, etc.) tissue.

2. I am an internationally recognized scientist and am presently Chief Scientific Officer and member of the Executive Board at Epigenomics, Berlin, Germany. I received a Ph.D. degree from the University of Bonn in 1989, and prior to joining Epigenomics have been employed as a post-doctoral researcher at Tufts University and the Max-Planck Institute for Molecular Genetics.

3. I am an author or co-author of many peer-reviewed research articles (representative articles are attached as APPENDIX A) in the field of methylation analysis and my research has been presented at national and international meetings.

4. In my capacity as Director of Research and Development at Epigenomics, I am well versed in all aspects of methylation analysis including bisulfite based technologies and methylation of relevant diagnostic and prognostic markers, and analysis of differential methylation of CpG dinucleotide residues in genomic DNA, and with identifying and characterizing statistically significant trends and correlations relating to DNA methylation in normal and diseased tissues, including cancer tissues and transformed cell lines.

5. I am familiar with particular aspect of the Office Action from the USPTO dated 06 June 2006 in this case, and understand that one or more claims of the above-referenced patent application remain rejected under 35 U.S.C. § 112, ¶1, on the alleged grounds that the claimed invention is not described in such a way as to enable one skilled in the relevant art to make and/or use the invention as claimed. Specifically, the Examiner states that the specification fails to teach that *any* CpG sequences of the SEQ ID NOS:46 and 47-containing islands would have the claimed diagnostic or prognostic utility, but rather only enables such utilities for genomic sequences within a more limited region of the CpG island (i.e., SEQ ID NOS:46 and 47), and that it would require undue experimentation to practice the invention with respect to the entire CpG island. Therefore, it appears that the Examiner is alleging that there is no predictable correlation between the methylation state of CpGs within SEQ ID NOS:46 and 47 and those of the larger respective CpG islands, and therefore that it is unpredictable that such coordinately methylated CpG island sequences are indicative, absent unpredictable and undue experimentation. I understand that the biological and chemical arts are generally regarded as relatively unpredictable, and that a patentable diagnostic utility in the present sense requires a reasonable correlation between methylation (or coordinate methylation) and disease states.

6. Specifically, I understand that a correlation between methylation of particular sequences and disease states has been reasonably made in the present specification, but that the Examiner is questioning whether it is reasonably predictable that CpG dinucleotide sequences within the complete subject CpG island are coordinately methylated. In the present prosecution for example, the Examiner has urged (citing Toyota et al.; attached hereto) that the art does not support the idea that all contiguous CpG islands are associated with cancer, because the CpG region upstream of, for example, CACNA1G appears to behave independently, and therefore,

since the art provides examples where CpG islands act in predictable ways (applicant) and examples where CpG islands act independently [Toyota, as construed by the Examiner], it is unpredictable whether the instant CpG islands act in a predictable or independent manner, and therefore despite applicants' correlative showing with respect to particular sequences, it is unpredictable that contiguous coordinately methylated regions are associated with disease.

7. *First*, while Toyota does teach examples where CpG islands act independently of each other, this is not the relevant question. The relevant question is whether the CpG dinucleotide sequences within a given CpG island behave *coordinately*. Here, the teachings of Toyota are in agreement with the instant Applicants' position. Specifically, Toyota initially describes/defines a large GC-rich 4Kb region, and divides region into 8 subregions. However, Toyota further notes that this region is considerably larger than typical CpG islands, and explicitly concludes that "with regards to hypermethylation in cancer, the CpG-rich region upstream of CACNA1G appears to be composed of two CpG islands that behave independently"; namely, Toyota concludes that "methylation of MINT 31 appears to be independent of methylation of CACNA1G, suggesting that they are two distinct CpG island regulated by different mechanisms." Significantly, therefore, Toyota teaches that while different CpG islands within a gene area can behave differently or independently, the particular CpG islands themselves behave coordinately so that methylation status of a subregion of the CpG island would be expected to reasonably correlate with the behavior of the entire CpG island. Therefore, Toyota like the vast bulk of art in this area (including Lapidus et al., cited by the Exainer), is fully consistent with the teachings of the present invention which teach that the CpG dinucleotides within a given contiguous CpG island are coordinately methylated.

8. *Second*, as to complex methylation patterns, that may be alleged by the Office Action, I bring to your attention a set of data (see attached paper by Eckhardt et al., *Nat Genet.* 2006 Dec;38(12):1378-85. Epub 2006 Oct 29) relevant to this discussion that has been generated by the company I work with (Epigenomics) in collaboration with one of the largest sequencing institutes in the world (Sanger Center). The aim of the collaboration was to analyze methylation (using bisulfite sequencing) in CG rich regions across chromosomes to provide a methylation map of the human genome (at least of the CPG rich regions thereof). To date, these data comprise methylation data of 3 complete human chromosomes (22, 20, and 6) for a variety of different tissues and cell types. Based on these data, for methylation patterns within CpG dense regions (*e.g.*, such as the present ESR1 CpG island) methylation is typically found to be either present for all methylatable cytosines or none. This methylation characteristic or pattern is

referred to in the art as "co-methylation" or "coordinate methylation." The findings of this paper support a "significant correlation" of comethylation over the distance of at least 1,000 nucleotides in each direction from a particular determined CpG (see, *e.g.*, page 2, column 2, 1st full paragraph, of attached Eckhardt et al publication document). Furthermore, such co-methylation forms the basis for long-standing common methods such as MSP (and particular MethyLight™ embodiments) that rely on such co-methylation (*e.g.*, the primers and/or probes each typically encompass multiple CpG sequences), and has now been further confirmed as part of the Eckhardt et al data. Therefore, in view of the teachings of the present specification, there is a reasonable correlation between the claimed coordinately methylated sequences, and the recited disease state(s). Moreover, such co-methylation was encompassed by Applicants' original conception.

9. I further declare that all statements made herein of my own knowledge are true and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code.

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Kurt Berlin

APPENDIX A

(Representative methylation-related peer-reviewed articles by Kurt Berlin)

Adorjan P, Distler J, Lipscher E, Model F, Muller J, Pelet C, Braun A, Florl AR, Gutig D, Grabs G, Howe A, Kursar M, Lesche R, Leu E, Lewin A, Maier S, Muller V, Otto T, Scholz C, Schulz WA, Seifert HH, Schwope I, Ziebarth H, Berlin K, Piepenbrock C, Olek A
Tumour class prediction and discovery by microarray-based DNA methylation analysis.
Nucleic Acids Res. 2002 Mar 1;30(5):e21.
PMID [11861926]

Cottrell SE, Distler J, Goodman NS, Mooney SH, Kluth A, Olek A, Schwope I, Tetzner R, Ziebarth H, Berlin K
A real-time PCR assay for DNA-methylation using methylation-specific blockers.
Nucleic Acids Res. 2004 Jan 13;32(1):e10.
PMID [14722226]

Tost J, Schatz P, Schuster M, Berlin K, Gut IG
Analysis and accurate quantification of CpG methylation by MALDI mass spectrometry.
Nucleic Acids Res. 2003 May 1;31(9):e50.
PMID [12711695]

Berlin K, Gut IG
Analysis of negatively 'charge tagged' DNA by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
Rapid Commun Mass Spectrom. 1999;13(17):1739-43.
PMID [10455243]

Eckhardt F, Beck S, Gut IG, Berlin K
Future potential of the Human Epigenome Project.
Expert Rev Mol Diagn. 2004 Sep;4(5):609-18.
PMID [15347255]

Rakyan VK, Hildmann T, Novik KL, Lewin J, Tost J, Cox AV, Andrews TD, Howe KL, Otto T, Olek A, Fischer J, Gut IG, Berlin K, Beck S
DNA methylation profiling of the human major histocompatibility complex: a pilot study for the human epigenome project.
PLoS Biol. 2004 Dec;2(12):e405. Epub 2004 Nov 23.
PMID [15550986]

Yang H, Chen CM, Yan P, Huang TH, Shi H, Burger M, Nimmrich I, Maier S, Berlin K, Caldwell CW

The androgen receptor gene is preferentially hypermethylated in follicular non-Hodgkin's lymphomas.
Clin Cancer Res. 2003 Sep 15;9(11):4034-42.
PMID [14519624]

Field JK, Liloglou T, Warrak S, Burger M, Becker E, Berlin K, Nimmrich I, Maier S
Methylation discriminators in NSCLC identified by a microarray based approach.
Int J Oncol. 2005 Jul;27(1):105-11.
PMID [15942649]

Guo J, Burger M, Nimmrich I, Maier S, Becker E, Genc B, Duff D, Rahmatpanah F, Chitma-Matsiga R, Shi H, Berlin K, Huang TH, Caldwell CW
Differential DNA methylation of gene promoters in small B-cell lymphomas.
Am J Clin Pathol. 2005 Sep;124(3):430-9.
PMID [16191512]

Eckhardt F, Lewin J, Cortese R, Rakyan VK, Attwood J, Burger M, Burton J, Cox TV, Davies R, Down TA, Haefliger C, Horton R, Howe K, Jackson DK, Kunde J, Koenig C, Liddle J, Niblett D, Otto T, Pettett R, Seemann S, Thompson C, West T, Rogers J, Olek A, Berlin K, Beck S
DNA methylation profiling of human chromosomes 6, 20 and 22.
Nat Genet. 2006 Oct 29;.
PMID [17072317]